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Greater than 80% of breast cancers are derived from epithelial cells. Epithelial cells are highly polarized, with separate apical and basolateral surfaces facing the lumen and adjacent cells. In epithelial tumors the degree of malignancy is correlated with extent of loss of polarity. Mammary cells can be transformed to varying degrees by regulated expression of various oncogenes. For instance, c-Jun over-expression gives a reversible transformation and loss of polarity, while sustained c-Fos overexpression gives an irreversible, more severe transformation and loss of cell polarity. The goal of this proposal is to observe the effects of differentiation, de-differentiation, and transformation on polarized membrane traffic in mammary epithelial cells. I have been investigating the hyothesis that modulation of epithelial polarity by TGFα and/or *c-myc* will affect the ability of mammary cells to correctly transport proteins from the TGN to the apical and basolateral PMs and during transcytosis, and will correspondingly affect expression of syntaxins. I am determining determine the effects of TGFα, *c-myc*, and glucocorticoids (alone or in combination) on polarized traffic from the TGN and transcytosis. I am examining the effects of these treatments on expression and localization of syntaxins 2, 3 and 4.

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Introduction

Greater than 80% of breast cancers are derived from epithelial cells. Epithelial cells are highly polarized, with separate apical and basolateral surfaces facing the lumen and adjacent cells, respectively. The apical and basolateral surfaces contain very distinct protein compositions, which are essential to the proper functioning of the cell. The establishment and maintenance of cell polarity requires that proteins be sent to the correct surface. Epithelial cells use two pathways to send proteins to the correct surface: direct delivery from the TGN to the surface or transcytosis. A great deal has been learned about polarized membrane trafficking in certain model epithelial cells, such as Madin-Darby canine kidney cells. In contrast, although the polarized steady state distribution of several proteins has been determined in normal and transformed mammary cells, very little is known about the pathways and mechanisms used for polarized trafficking in mammary cells.

Mammary cells undergo a physiological cycle of growth, differentiation, lactation, and involution. During transformation mammary cells lose polarity, and the extent of loss of polarity correlates with the degree of malignancy. The physiological and oncogenic changes in mammary cell polarity can be studied using highly differentiated cultured mammary epithelial cell lines, such as the 31EG4 cell clone. Glucocorticoids cause an increase in polarity. In contrast, exposure to TGFβ or over-expression of oncogenes (c-Fos or c-Jun) cause a spectrum of loss of polarity. For instance, c-Jun over-expression causes a reversible, partial loss of polarity, whereas over-expression of c-Fos causes a severe and irreversible loss of polarity. In contrast, exposure to TGFβ acts primarily to antagonize glucocorticoids, and physiologically is probably involved in involution of the mammary gland.

In this grants have been studying the normal pathways used for polarized membrane traffic in well-differentiated cultured mammary epithelial cells. We will then move on to the effects of glucocorticoids, addition of TGFβ, or overexpression of c-Jun or c-Fos on these pathways. We will examine the effects of these manipulations on expression and localization of syntaxins, which are a family of proteins that appear to control the specific targeting of vesicles to either the apical or basolateral surfaces of the cell. Finally, we will determine if overexpression of different syntaxins can correct the defects in membrane traffic caused by the manipulations that disrupt cell polarity. We have also been examining the roles of Pak1 and raf on cell polarity and membrane traffic.

The majority of breast cancers are epithelial in origin. 75% are derived from the ductal epithelium, while 5-10% are derived from lobular epithelial cells (1-3). Simple epithelia form a layer which acts as a highly selective barrier between the lumen of the duct or lobule and the adjoining and underlying cells. To achieve this the structure of epithelial cells is highly polarized. This is manifested in the maintenance of two distinct plasma membrane (PM) domains: an apical domain comprising the lumenal surface, and a basolateral domain contacting adjoining and underlying cells. These two PM domains have very different protein compositions. Generation and maintenance of cell polarity is essential to epithelial function. This requires sorting PM proteins to the correct apical or basolateral surface. The polarity of mammary cells changes as they undergo the physiological cycle of growth, differentiation, and involution. An early hallmark of epithelial transformation is the loss of functional and morphological polarity.

In recent years we have learned a great deal about how PM proteins are sent to the apical or basolateral domain. Almost all of this work has been in non-mammary epithelial cells, especially the Madin-Darby canine kidney (MDCK) cell line (4-6). Much less is known about how the pathways and mechanisms of polarized traffic are altered as cells undergo physiologic differentiation and de-differentiation or as the cells undergo transformation. The fundamental innovation of this proposal is to apply our advances in studying polarized membrane traffic to the alterations in mammary cell polarity caused by a variety of physiological (e.g. exposure to glucocorticoids or TGFβ) or oncogenic factors (over-expression of c-Jun or c-Fos oncogenes).

Alterations in polarized membrane traffic are highly relevant to breast cancer for several reasons. The delivery of growth factors and their receptors to the cell surface must be controlled. Mammary epithelial cells are responsive to many growth factor receptors at their basolateral PM, such as EGF, TGF-α, TGF-β and PDGF. They can also secrete these factors from their apical cell surface. Correct targeting of these growth factors is essential to control their growth (7-9) . On another level polarized trafficking is

important because many growth factors are first formed as larger proteins, and their proteolytic products are considered the active form. However, if not processed properly, the membrane bound protein is still active. Clearly, proper expression and localization of the processing enzymes is critical.

In general in epithelial tumors the degree of malignancy is correlated with extent of loss of polarity. Cultured mammary cells can be transformed to varying degrees by regulated expression of various oncogenes. For instance, c-Jun over-expression gives a reversible transformation and loss of polarity, while sustained c-Fos overexpression gives an irreversible, more severe transformation and loss of cell polarity.

Understanding how polarized membrane changes during physiological and oncogenic processes has several clinical implications. Like colon and other cancers, it is likely that breast cancers undergo a progression of increasing malignancy. With modern diagnostic techniques many very early breast cancers (e.g. ductal carcinoma in situ) are now being detected. It is difficult, yet vitally important, to predict which of these will progress to more serious malignancies. In this grant proposal we will examine how a spectrum of physiological and oncogenic factors alters the pathways and machinery for membrane traffic. We will focus on the syntaxins, a family of proteins that appear to specify correct delivery to the apical or basolateral surfaces. We predict that alterations in pathways and machinery (e.g. expression of various syntaxins) may correlate with the degree of malignancy. Therefore, it may ultimately be possible to examine breast tumor biopsy specimens for the expression of particular syntaxins (or other elements of the polarized trafficking machinery) and use this to predict which tumors will progress. Similarly, it is difficult, yet crucial, to predict how any given tumor will respond to various treatment modalities. Again, examining biopsy specimens for polarized membrane traffic and expression of syntaxins may be very informative as a prognostic or responsive factor.

Finally, and most speculatively, if loss of expression of syntaxins is part of the mechanism of loss of polarity and increased malignancy, then it may be possible to over-express exogenous syntaxins, and thereby partially correct the defect.

Body

The goal of this proposal is to observe the effects of differentiation, de-differentiation, and transformation on polarized membrane traffic in mammary epithelial cells. Epithelial cells contain separate apical and basolateral plasma membranes (PM), with very different protein and lipid compositions (Fig. 1, addendum B). Generating and maintaining PM polarity is essential for the proper growth and function of an epithelium. Loss of polarity is an early and crucial step in the development of malignancy. Polarized expression is especially important for growth and differentiation factors and their receptors. For example, the mistargeting of an apically secreted growth factor to the basolateral PM can result in inappropriate stimulation of its cognate receptor (7, 12). Alternatively, if the delivery of a receptor to the cell surface is blocked and pools of receptor form on internal membranes, interaction with its ligand would again result in an unregulated activation of the receptor (13, 14). During lactation several growth hormones are synthesized and secreted into the milk, including the IGF-family, epidermal growth factor, transforming growth factors (TGF) α and β, tumor necrosis factor, basic fibroblast growth factor and platelet derived growth factor. An autocrine growth response, whereby a tumor cell both expresses and is stimulated by a mitogenic factor, may contribute to the tumorigenicity of neoplastic cells (8, 9).

To establish and maintain their polarity, epithelial cells must send PM proteins to the correct apical or basolateral PM (4-6). Epithelial cells utilize two major pathways to send proteins to the correct PM. Newly made PM proteins can be sent from the Golgi and trans-Golgi network (TGN) to either the apical or basolateral surface (Fig. 2). Alternatively, proteins can be sent first to one PM, generally the basolateral. The proteins can then be endocytosed and transcytosed across the cell to the apical PM (Fig. 3). Transcytosis to the apical PM is the only mechanism for apical delivery found in all epithelial cells and in some cell types it is the only mechanism for apical delivery. The steady state localization of a protein at a particular PM is the result of the rate of delivery of the protein to that surface and its rate of removal and/or degradation.

Although the steady state localization of several PM proteins has been examined in normal and transformed mammary cells, very little is known about the pathways and mechanisms used for their delivery. In contrast, by far the most work on polarized membrane traffic has been carried out on MDCK cells.

A major discovery in recent years is that almost all intracellular membrane traffic events use a common machinery for membrane fusion (15-20). Core elements of this machinery include soluble components, NSF and αSNAP . Transport vesicles contain v-SNAREs (vesicular SNAP receptors), such as VAMP-1 . The transport vesicles fuse with target membranes, which contain t-SNAREs (target SNAREs). For instance, in neurons, synaptic vesicles containing VAMP-1 fuse with the plasma membrane, containing t-SNAREs. The t-SNAREs consist of two subunits, syntaxins and SNAP-25. (SNAP-25 is unrelated to αSNAP.) A key event in fusion of vesicles is the binding of the v-SNARE to the t-SNARE via their cytoplasmic domains. Both v-SNAREs and t-SNAREs (syntaxins) consist of gene families. It was originally observed in yeast that each step in membrane traffic (e.g. ER to Golgi, Golgi to vacuole, or Golgi to PM) utilized an exclusive pair of v- and t-SNAREs. This led to the hypothesis that the correct pairing of v-and t-SNAREs conferred **specificity** upon vesicle fusion, so that a vesicle could not fuse with the wrong target.

Polarized epithelial cells are an excellent system in which to examine the possible role of SNAREs in conferring specificity, as they contain several classes of vesicles traveling from either the TGN or endosomes to the apical or basolateral PMs. K. Simon's group investigated this by reconstituting traffic in permeabilized MDCK cells (21). Surprisingly, they found that transport from the TGN to the apical PM of at least one protein, influenza virus hemagglutinin (HA), did not require NSF. (22, 23). Although these results indicate that the TGN to apical PM pathway of HA uses a non-NSF/SNARE mechanism, molecules can also reach the apical PM by transcytosis. We therefore investigated whether transcytosis of pIgR to the apical PM would also use a non-NSF/SNARE pathway (24). We replicated Simon's permeabilized MDCK cell system and used it to reconstitute transcytosis. We found that transcytosis of dIgA to the apical PM uses a NSF/SNARE dependent mechanism. Thus, delivery to the apical PM can be either NSF/SNARE dependent or /independent .

The finding that delivery to the apical PM (transcytosis) and the basolateral PM both use NSF/SNAREs leads to the question of whether specificity in apical/basolateral targeting depends on using different SNAREs. Five isoforms of the t-SNARE, syntaxin, occur at the PM of mammalian cells (25). Two of these, syntaxins 1a and 1b, are neuron specific, whereas syntaxins 2, 3, and 4 are abundant in epithelial organs, e.g. liver, kidney, and intestine. In collaboration with Mark Bennett (who discovered syntaxins in R. Scheller's lab and now has his own lab at UC Berkeley), we investigated the location of all of these syntaxins in MDCK cells. Dr. Bennett has used GST fusions with clones of rat syntaxins 1-4 to produce affinity-purified, isoform specific antibodies against each syntaxin. We used these antibodies on western blots of MDCK cells and detected syntaxins 2, 3, and 4.

To investigate the localization of these syntaxins in MDCK cells, Steve Chapin in our lab separately transfected syntaxins 2, 3 or 4 into MDCK cells and isolated cell lines stably expressing individual syntaxins (together with pIgR). We examined the location of each isoform by confocal immunofluorescent microscopy. Syntaxin 2 was primarily on the apical PM, with some staining of the basolateral PM. Syntaxin 3 was on the apical PM and on vesicles in the apical region of the cytoplasm. This agreed completely with the faint staining of the endogenous syntaxin 3. Syntaxin 4 was entirely on the basolateral PM. We conclude that each syntaxin has a unique localization. The localization of syntaxins 2 and 3 at the apical PM is consistent with our finding that at least some membrane traffic to the apical PM uses SNAREs. Most importantly, syntaxins 3 and 4 have a completely non-overlapping distribution and must serve different traffic pathways.

We found that overexpression of syntaxin 3 inhibits TGN to apical delivery. This result suggests that TGN to apical transport of some proteins use an NSF/SNARE dependent mechanism. The other syntaxins had no effect on apical delivery. In contrast to the apical PM effects of overexpression of syntaxin 3, overexpression of syntaxin 4 had an effect on basolateral traffic, e.g. it inhibited recycling of dIgA back to the basolateral surface. (We have not yet analyzed the effects of syntaxin 2 overexpression, but given its location at both the apical and basolateral surfaces, it may be involved in transcytosis between these surfaces.) We do not know why overexpression of syntaxins 3 and 4 had inhibitory effects, but it should be noted that overexpression of syntaxin 1 in insulinoma cells similarly blocks regulated secretion (26), suggesting that the inhibition of membrane traffic by syntaxin overexpression may be a general phenomena. A likely explanation for these results is that the high level of the overexpressed syntaxin drives formation of the syntaxin complex with a v-SNARE, and thereby slows the disassembly of this complex needed for fusion to proceed. It is highly unlikely that the observed effects on membrane traffic are due to nonspecific artifacts of overexpression. The effects of each syntaxin are highly specific to either the apical or basolateral PM. Syntaxin 3 overexpression had no effect on the basolateral PM, while syntaxin 4 had no effect on the apical. The syntaxins are the first family of molecules that are part of the membrane fusion machinery, whose isoforms are differentially localized to the apical or basolateral PMs of polarized cells and whose overexpression has differential effects on polarized membrane traffic. They are therefore the leading candidates for containing at least part of the information needed for vesicular targeting.

When proteins that are sorted to the apical or basolateral PMs of epithelial cells are expressed in non-polarized fibroblasts, these proteins are still sorted in the TGN into cognate apical or basolateral vesicles (27). Apparently, as these cells lack distinct apical and basolateral PMs, the vesicles all fuse with the one common PM. Kai Simons has therefore suggested that the major difference between polarized epithelial cells and non-polarized cells is the presence or absence of the machinery for vesicles to fuse with the correct PM. Our results clearly indicate that syntaxins are part of the machinery needed for this correct targeting. We hypothesize that the repertoire of expressed syntaxins will be diminished in cells that are de-differentiated or transformed.

The mammary gland cycles through a well-known series of growth, differentiation, lactation, and involution steps. As an in vitro model, we will use a clonal population selected from the cell line IM-2, derived from spontaneously immortalized, nontumorigenic mouse mammary cells. IM-2 represents a heterogeneous cell population consisting of both epithelial (31E) and fibroblastic (30F) cells. When cultured in the presence of the lactogenic hormones, IM-2 cells are induced to secrete large amounts of β-casein. When the epithelial cells, 31E, were grown on a permeable support they can be induced to

differentiate by the addition of prolactin and glucocorticoid (28). 31E cells form a well polarized monolayer, as shown by EM studies and the establishment of a transepithelial electrical resistance (TER) when grown on permeable supports. Lactate and laminin are released from the basal surface, whereas β-casein is secreted from the apical cell surface (29). 31E cells were subcloned further to generate a more stable homogeneous cell line designated, 31EG4 (30). Recihmann's group isolated virtually identical subclones, IM-Ep-1 and EpH4 (31, 32).

Subcloning of 31EG4 and much of their further characterization described below was performed by Karen Singer Zettl [now Karen Singer], as a graduate student in Prof. Gary Firestone's laboratory at Univ. of California, Berkeley. Dr. Singer is now a postdoctoral fellow in my laboratory, and has been invaluable in preparing this grant proposal, as well as setting up the 31EG4 system. Dr. Singer is now pursuing research that is complementary to this grant proposal, but is non-overlapping, as it considers the effects of TGFα and c-Myc on polarized membrane traffic.

Using 31EG4 cells, a new role for glucocorticoids in mammary development was recently defined. Cell monolayers grown on permeable supports in the presence of dexamethasone, a synthetic glucocorticoid, resulted in a 64-fold increase in the transepithelial electrical resistance and a two fold increase in the expression of the tight junction protein ZO-1 (32). Additional experiments on the effects of glucocorticoids on 31EG4 cells demonstrated that in the presence of prolactin the functional polarity of the cell surface was induced (37). This was the first demonstration in the induction of differentiation resulting in the increased cell polarity for a monolayer grown on a permeable support, which makes this an ideal model system.

The synergistic transforming potential of TGFα and *c-myc* were convincing demonstrated by Dickson and colleagues (12). When single transgenic mice for TGFα and *c-myc* were mated, the homozygous TGFα /*c-myc* mice **all** developed mammary tumors. Tumors developed synchronously in both males and females at 66 days. Independently, c-myc expression in transgenic female mice resulted in approximately 50% tumor formation, although much later in life. No tumors were found in male mice expressing *c-myc* or in either sex expressing TGFα. When expressed in vitro in IM-2 derived cells, *c-myc* deregulated proliferation, leading to an increased number of cells. However, there was no change in monolayer TER, or in the localization of apical or basolateral protein markers (33, 34). It was found that the localization of ZO-1 and E-cadherin in 31EG4 cells is also unchanged in cells grown with insulin or with insulin and dexamethasone (32). However, the functional polarity of the Na^+/H^+ exchanger became exclusively basolateral in the presence of glucocorticoids. I propose that the effects of *c-myc* expression may only be uncovered by investigating the additional properties of protein delivery from the TGN to the cell surface, the ability of the cell to regulate intracellular trafficking, and changes in syntaxin expression and localization.

TGFα binds the EGFR to initiate a signaling cascade to stimulate cell growth. TGFα was demonstrated to be responsible for an autocrine growth response in Con8 cells, a rat mammary tumor cell line derived from a 7,12-dimethylbenz(α)anthracene-induced rat mammary adenocarcinoma. Growth of Con8 cells was strongly inhibited by dexamethasone (39). This was later shown to be due to the inhibition of TGFα secretion (7). TGFα is a natural ligand for the EGF receptor. Exogenous TGFα was able to restore growth, even when Con8 cells were grown in the presence of dexamethasone (14). Glucocorticoids inhibit Con8 cell growth by altering the output of TGFα. When added to cells grown on permeable supports, dexamethasone affected cell polarity, such that the exogenous addition of TGFα to the basolateral, but not the apical membrane domain of Con8 cells stimulated cell growth (40). The mechanism of TGF-α action on Con8 cells in the presence of dexamethasone was post-transcriptional, and did not require DNA synthesis. Soloman and colleagues found that expression of antisense TGF-α in the estrogen responsive mammary cell line MCF-7 prevented the estrogen dependent stimulation of growth (41). The effect of TGFα on cell polarity has not been investigated in any of the IM-2 epithelial cell derivatives.

In summary, alterations in mammary epithelial cell polarity in response to glucocorticoids (increased polarity), *c-myc* (no change in polarity), and TGFα (dominant loss in cell polarity) reflect a spectrum of

differentiation and polarization of the mammary gland during differentiation and tumorigenesis. How all these events are reflected in polarized trafficking is what I am addressing.

I have been investigating the hyothesis that modulation of epithelial polarity by TGFα and/or *c-myc* will affect the ability of mammary cells to correctly transport proteins from the TGN to the apical and basolateral PMs and during transcytosis, and will correspondingly affect expression of syntaxins. I am determining determine the effects of TGFα, *c-myc*, and glucocorticoids (alone or in combination) on polarized traffic from the TGN and transcytosis. I am examining the effects of these treatments on expression and localization of syntaxins 2, 3 and 4. I am analyzing if over-expression of syntaxins can alter the effects of these treatments.

I have started by examining membrane traffic in control 31EG4 cells, which form a well polarized monolayer on permeable Transwell supports. I will use the same basic strategy and assays Dr. Mostov developed for analysis of membrane traffic in MDCK cells . He has published details of all these methods, using both transfected pIgR and endogenous markers (E-cadherin for the basolateral PM, DPP IV for the apical PM) (6, 42, 43). For instance, delivery of pIgR or E-cadherin from the TGN to the basolateral surface is measured by metabolically labeling pIgR and E-cadherin with a 10 min pulse with ^{35}S-Cys. Cells are then chased for various periods of up to 60 min. The arrival of pIgR or E-cadherin at the basolateral surface is then determined by labeling proteins at the basolateral PM with a membrane impermeant biotinylation reagent (sulfo-NHS-biotin). (A duplicate set of filters is used to measure mis-delivery to the apical PM). The pIgR or E-cadherin are then immunoprecipitated, and the immunoprecipitates reprecipitated with streptavidin-agarose. These are then analyzed by SDS-PAGE and fluorography. This procedure measures the rate and accuracy of delivery of a protein to the basolateral PM. To measure delivery to the apical PM I can use DPP IV or transfected pIgR with a mutant basolateral signal, which is sent directly to the apical surface. A theoretical problem is that when cells lose their tight junctions, the sulfo-NHS-biotin can leak between cells and non-specifically label both surfaces. However, in preliminary experiments, Dr. Mostov's group has found that if the labeling with biotin is performed for short periods (e.g. 5 min) at 4°C), this is a very minor problem and does not interfere with our analysis. To measure transcytosis, I am allowing the cells expressing the pIgR to endocytose radioiodinated IgA (^{125}I-IgA) from the basolateral surface for 5 min. The cells are then washed and the subsequent release of ^{125}I-IgA into the apical medium (transcytosis) and basolateral medium (recycling) is measured over a 120 min period.

I am using the spectrum of treatments described above to perturb polarity to various degrees and examine the effects on traffic. For glucocorticoids, I use dexamethasone (dex; 1 μM), which is expected to increase polarity. Addition of TGFα (l0 ng/ml) causes a loss of TER in Con8 cells, but I will investigate this in 31EG4 cells. (Dr. Rik Derynck has agreed to serve as a consultant to this project; see enclosed letter). Given the antagonist effects of TGFα and glucocorticoids, I am trying different combinations including: no dex, or dex for 48 hours, then the addition of TGFα either basolaterally or apically. TGFα and *c-myc* are synergistic, so that experiments will be designed first with the over-expression of *c-myc* alone, then TGFα added to either the apical or basolateral media. The presence of c-myc is not reported to decrease TER, therefore it will interesting to observe how they differ in their response as compared to dexamethasone. Glucocorticoids are growth inhibitory, while *c-myc* is growth promoting. *C-myc* will be expressed from the tetracycline responsive transactivator system developed by Bujard and colleagues. The original version of this system involves sequentially transfecting the cells with two plasmids, encoding first the tetracycline sensitive transactivator, and then the gene of interest (e.g. *c-myc*) under the control of a promoter that is responsive to this transactivator. Cells are grown in the presence of tetracycline, which represses the expression of the gene of interest. When tetracycline is removed, the gene of interest is turned on. K. Mostov's group has successfully used this system in MDCK cells to obtain very tightly regulated and highly inducible expression of a variety of genes.

In all cases I use confocal immunofluorescence microscopy of several marker proteins (E-cadherin, ZO-1, etc.) and transepithelial electrical resistance to verify that the changes in polarity are consistent with previously reported results. I am using the array of assays described above to determine the effects on membrane traffic.

During the development of polarity in MDCK and other epithelial cells, TGN to apical delivery is the last process to develop and is indicative of the fully polarized phenotype. In contrast, transcytosis to the apical surface is used during an intermediate stage of development of polarity, while TGN to basolateral delivery is found even in non-polarized cells. .

Transport to the apical surface in both the TGN to apical and transcytotic pathways is stimulated by several pharmacological agents that raise cAMP (e.g. forskolin), intracellular free calcium (thapsigargin), or activate protein kinase C (PKC, phorbol myristate acetate) (4-6). In contrast, transport to the basolateral surface is largely unaffected by these agents. It has recently been shown that even in "non-polarized" fibroblasts, transport to the PM of normally apical proteins is similarly stimulated by these agents, while transport to the basolateral surface is not (29). Therefore, distinct populations of apical and basolateral vesicles exist even in fibroblasts. I am using these agents on mammary cells that have been depolarized by by the manipulations described above and examine the effects on PM delivery of our apical and basolateral marker proteins. I expect that even the most depolarized mammary cells will retain separate apical and basolateral vesicles that will show a differential response to these agents.

FIGURES.

Fig. 1. Epithelial cell polarity

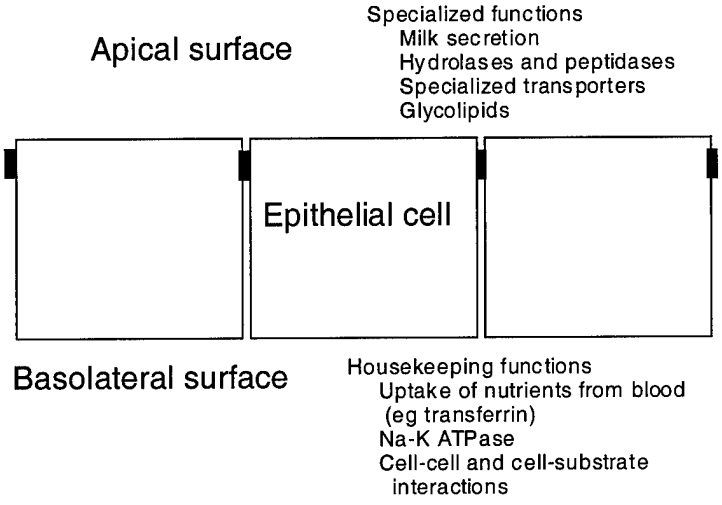


Fig 2. Polarized Sorting from TGN

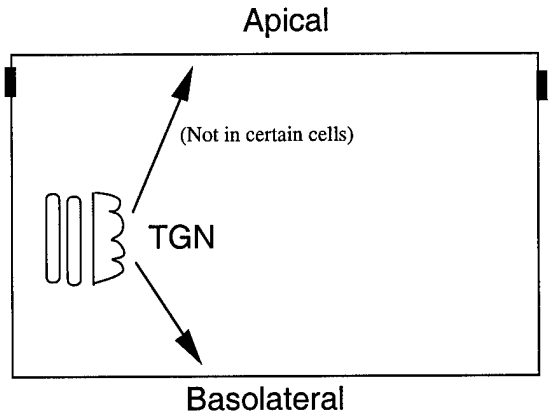


Fig. 3. Transcytosis and Polarized Sorting in Endosomes

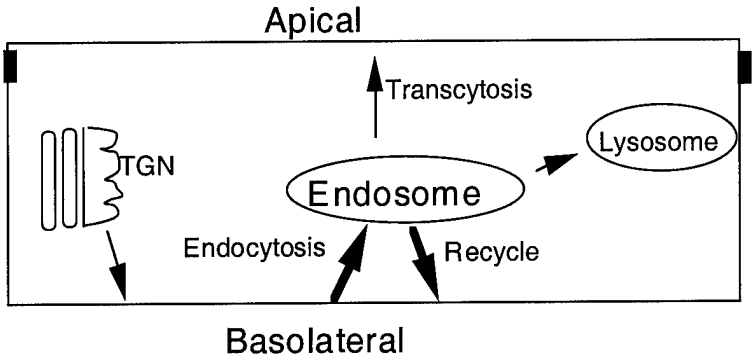
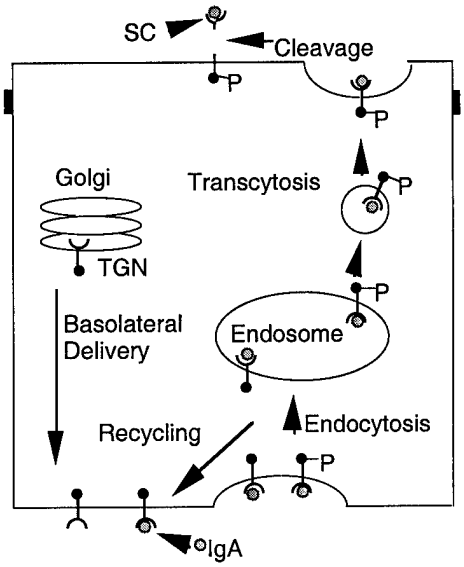


Fig. 4. Pathway of the pIgR..



Conclusions:

We have begun a systematic examination of the pathways of membrane traffic in normal and transformed mammary epithelial cells. This work is laying the groundwork for fullfillment of the complete research program of this grant, to understand how membrane traffic is altered in mammary cancer and how it may be possible to restore it to normalcy.

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APPENDIX: ABBREVIATIONS

dIgA	dimeric IgA
DPP IV	dipeptidyl peptidase IV
MDCK	Madin-Darby canine kidney cells
NSF	N-ethyl maleimide sensitive factor
pIgR	polymeric immunoglobulin receptor
PM	plasma membrane
SC	secretory component
α-SNAP	soluble NSF attachment protein
SNAP-25	synaptosomal associated protein of 25kD
TGFα	transforming growth factor α
TGFβ	transforming growth factor β
TER	transepithelial electrical resistance
TGN	trans-Golgi network
t-SNARE	target SNAP receptor;.
v-SNARE	vesicle SNAP receptor
WT	wild-type